MACROSCOPIC PHOTOGRAPHY OF THE INTESTINE AS A DIAGNOSTIC TOOL IN EXPERIMENTAL GASTROENTEROLOGY

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During the past four years use of the dissecting microscope for morphologic analysis of intestinal biopsies has been of considerable value in the characterization of normal intestinal villous forms, and in the diagnosis of coeliac disease, idiopathic steatorrhea, and other malabsorption syndromes, [McCarthy et al, 1964, Brackenbury and Stewart, 1963, Holmes et al, 1961a, 1961b]. Such a technique has at times proven to be superior to conventional histologic procedures, since cross sections of ridges in the latter technique can produce artifacts that appear like villi, thus hindering an evaluation of the degree of villous atrophy [McCarthy, et al, 1964]. However, reports have not appeared in which the dissecting microscope has been utilized routinely for diagnosis of intestinal pathologies in experimental animals. Although intestinal villi of the laboratory rat are grossly barely visible, conventional histologic techniques have been used for many years in experimental pathology of the gastrointestinal tract of the rat. Since the rat is commonly used in gastrointestinal experiments involving pyloric-ligation [Shay et al, 1945], restraint [Rossi et al, 1956, Brodie and Hanson, 1960], drug administrative [Segal, 1960, Pfeiffer and Gass, 1962], behavioral

[Sawrey and Weisz, 1956, Ader et al, 1960], and other procedures, the following experiments were conducted to determine 1, the normal villous structure for various regions of the rat intestine, and 2, whether macroscopic techniques can be used for pathologic diagnoses of the intestines of the rat.

Materials and Methods

Thirty-nine normal male Sprague-Dawley rats (150-300 g) were sacrificed by intracranial injection of alcohol. The entire intestinal tract was immediately removed and placed in warm 0.9% NaCl solution. One-inch segments from the duodenum, jejunum, ileum, and distal colon were dissected from the alimentary canal, everted, cleaned, and photographed. Cleaning of villi was accomplished by gentle shaking of the everted segment in 0.9% NaCl; excess debris was removed with a small forceps. The influence of starvation on villous forms was studied on rats fasted 72 hours (water ad libitum). The influence of pylorus ligation was studied on intestinal segments from animals subjected to the standard operation of Shay [1945] and sacrificed 15-17 hours after ligation.

Photography was accomplished with an E. Leitz Aristophot Macro Camera with a 4 in. × 5 in. format using a 24-mm or 42-mm Summar lens stopped down to the maximum f/stop of 12 for depth of field. A long bellows extension was used; initial negative magnifications were approximately 10 and 20. The specimen was immersed in a 0.9% NaCl solution in a petri dish. The clear glass dish allowed illumination of the specimen from both above and

below the level of the tissue. Since long exposures averaging 15 seconds were required, specimens were weighted down by a glass rod to prevent floating movements. Also, since lighting is extremely critical in macrophotography at 10 to 20 magnifications, the glass rod inserted through the everted gut segment facilitated parallel alignment of the specimen with the lens in order to provide even lighting. A Photovolt Meter, Model 501M, was used to determine exposures, and one or a combination of two 6 volt, 8 amp. Tyoda coil filament lamps with adjustable diaphragms were employed. The angle of light is very critical, especially in photography of the colon; in the present study the angle of light ranged from approximately 3° below the petri dish to approximately 20° above the specimen. Orthochromatic films (Ansco Commercial Ortho) were used in most instances, with an orthochromatic film of relatively high contrast for colon photography. Villous dimensions were determined photomicronometrically.

Results

Villous forms in normal rat duodenum (Fig. 1), jejunum (Fig. 2), and ileum (Fig. 3) were found to be quite consistent for corresponding regions in different rats, with an increasing spatulateness of villi from the duodenum to the ileum. This is in contrast to the marked variance in villous forms observed in corresponding intestinal regions of normal humans [Brackenbury and Stewart, 1963; Holmes et al, 1961a, 1961b], and to the

decreasing spatulateness of human villous forms from the duodenum toward the ileum. In general, rat duodenal, jejunal, and ileal villi resemble the "tongue and narrow leaf" forms characterized by Holmes et al [1961a], Brackenbury and Stewart [1963], and McCarthy et al [1964] for human tissue; the "finger like" and "ridge" forms described by these authors are uncommon in the rat. The width of villi from various regions of the normal rat small intestine varies from approximately 238-794 μ, and the thickness from approximately 81-238 μ. The height of villi is not measurable by this method. Normal colonic tissue in the rat is void of villi (Fig. 4), and is lined with crater-like pits, approximately 61-91 μ in diameter. These pits are the openings to the crypts of Lieberkühn, and are essentially invisible to the unaided eye.

During the course of various stress impositions, (starvation, pyloric ligation, gastric ulcer perforation) a few macroscopic alterations were notable in the intestinal villi. A slight flattening of duodenal villi occurred (Fig. 5) in a pylorus-ligated rat (approximately 40 hr. total pre- and post-surgical fasting). This phenomenon was markedly exaggerated (Fig. 6) in rats which had been fasted for 72 hours, but not subject to pylorus ligation. One rat, in which a rumenal ulcer perforated following pylorus ligation, developed a hemorrhagic duodenal ulcer that was grossly visible (Fig. 7). It is noteworthy that 1, this duodenal "stress ulcer" developed in a presumably acid-free environment (due to pylorus ligation) and 2, villous atrophy

was macroscopically apparent adjacent to the site of frank hemorrhage (Fig. 7). As indicated in Fig. 8, villous flattening and narrowing were evident in the jejunum following 72 hours fasting, but were less marked than in duodenal tissue. Ulcers were observed on one occasion in the colon of an untreated rat (Fig. 9). Although the etiology of the colonic ulcers is not known, it is interesting to compare their morphologic similarity to chronic human gastric ulcers [Ivy et al, 1950]. The colonic ulcers observed here in the rat and human gastric ulcers are both indurated with margins elevated by a marked proliferation of connective tissue.

Discussion

The present photographic technique is offered as a useful diagnostic tool for small animals in experimental gastroenterology. The macroscopic analysis of villous forms with the dissecting microscope provides a panoramic and informative means of appraising various pathologies of the intestine, and constitutes a useful adjunctive procedure for routine histologic studies. Diagnostic criteria relate to the degree and nature of villous atrophy, which appear to be notable in the rat intestine following exposure to various stressors, and resemble to a limited degree the villous atrophy observed in human idiopathic steatorrhea and coeliac disease [McCarthy et al, 1964; Brackenbury and Stewart, 1963; Rubin et al, 1960]. Thus, the dissecting microscope may prove useful in the

investigation of experimental steatorrhea, since this syndrome can be induced in the rat [Hoet and Eyssen, 1964]. The villous atrophy observed in the present starvation experiments concurs with the report of Sun [1926], who demonstrated shortening or disappearance of villi in starved mice, but contrasts with the report of Hooper and Blair [1958], which indicated a normal appearance of duodenal mucosa of starved rats.

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Figure Titles

Fig. 1.- Duodenum of normal rat. ×22

Fig. 2.- Jejunum of normal rat. Note increase in broadness from duodenal villi. ×30

Fig. 3.- Ileum of normal rat. Darkening of central regions of villi due to vascularity. ×50

Fig. 4.- Colon of normal rat. Note openings to crypts of Lieberkühn and small arteries. ×50

Fig. 5.- Duodenum of pylorus-ligated rat. Note slight flattening of villi not apparent in normal duodenum (Fig. 1). ×23

Fig. 6.- Duodenum of fasted (72 hr.) rat. Note marked flattening of villi. ×22

Fig. 7.- Duodenal "stress ulcer" in pylorus-ligated rat with perforated rumenal ulcer. Note dark central area (frank hemorrhage) and adjacent villus atrophy. ×22

Fig. 8.- Jejunum of fasted (72 hr.) rat. ×22

Fig. 9.- Ulcers in colon untreated rat. Note similarity to chronic, human gastric ulcer. ×24